E3S Web of Conferences **603**, 01030 (2025)
ISGST 2024
https://doi.org/10.1051/e3sconf/202560301030

Exploring the influence of carbon sources and salinity on the growth of microalgae

Xin Yi Choong[1,2], *Hui Ying* Teh[1,2], *Man Kee* Lam[1,2*], *Revathi* Raviadaran[1,2], *Yee Ho* Chai[1,2], *Inn Shi* Tan[3], *Henry Chee Yew* Foo[3], *Voon-Loong* Wong[4], and *Adrian Chun Minh* Loy[5]

[1]Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia
[2]HICoE-Centre for Biofuel and Biochemical Research (CBBR), Institute of Sustainable Energy, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia
[3]Department of Chemical and Energy Engineering, Faculty of Engineering and Science, Curtin University Malaysia, CDT 250, 98009, Miri, Sarawak, Malaysia
[4]School of Energy and Chemical Engineering, Xiamen University Malaysia, Selangor Darul Ehsan 43900, Malaysia
[5]Department of Chemical and Biological Engineering, Monash University, 3180 Victoria, Australia

Abstract. In the domain of microalgae cultivation, the selection of carbon source and salinity profoundly impacts the growth and metabolic activity of species like *Chlorella* sp. Carbon sources and salt serve as vital substrates, dictating not only biomass production but also shaping cellular processes essential for various applications, particularly as agricultural biofertilizers. This study investigated the impact of different carbon sources and varying concentrations of sodium chloride (NaCl) on the growth of *Chlorella* sp. It was found that CO_2 bubbling significantly improved microalgae growth, resulting in a notable 5.60% increase compared to cultivation with sodium bicarbonate. Within a span of 14 days, *Chlorella* sp. reached its peak biomass of 1.32 g/L ± 1.2% under CO_2 bubbling, outperforming $NaHCO_3$ cultivation, indicating a more efficient carbon utilization. Furthermore, the study revealed that *Chlorella* sp. achieved its highest biomass and lipid yield under CO_2 bubbling cultivation without the addition of NaCl (1.32 g/L ± 1.2% and 0.43 g/L ± 3.0 % respectively), while a NaCl concentration of 0.5 M yielded the highest lipid content (34% ± 1.8 %) but had relatively low lipid yield at 0.21 g/L ± 5.0%. This underscored the impact of NaCl stress on the growth and lipid content of *Chlorella* sp.

1 Introduction

In the forthcoming decades, the assurance of global food security rests heavily upon the attainment of agricultural sustainability. This is crucial for countries like Malaysia, which have experienced significant economic growth in the agricultural sector. Malaysia currently accounts for 26% of global production and 34% of global exports in palm oil production as of 2020 [1]. However, the historical focus on intensification in agriculture has precipitated

*Corresponding author: lam.mankee@utp.edu.my

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

environmental deterioration [2]. Practices such as fertilizer application, tillage, alterations in land use, and the cultivation of specific crop varieties heavily depend on natural resources. Collectively, these practices have engendered a troubling decline in biodiversity, especially among non-domesticated species [3]. While conventional agriculture has traditionally relied on chemical fertilizers to enhance crop yields, their adverse environmental effects have raised apprehensions [4]. Chemical fertilizers can diminish soil fertility, adversely impact beneficial organisms, and contaminate groundwater [5]. Consequently, biofertilizers are emerging as a promising alternative.

Additionally, the optimal growth and production of microalgae as biofertilizer are significantly influenced by environmental conditions [6]. The photoautotrophic cultivation of microalgae faces several challenges, particularly low biomass and lipid productivity. These limitations are primarily due to photo limitation, where high cell density reduces light penetration [7]. To produce microalgae biofertilizer, carbon source and salt concentration is important. Carbon is an important nutrient that maintains the microalgae growth and metabolism [8]. Microalgae can utilize various carbon sources (organic and inorganic carbon source) through different metabolic pathways, affecting their growth rates, biomass yield, and biochemical composition [8]. Increased availability of carbon from external carbon sources can lead to higher carbon fixation rates, potentially boosting the accumulation of biomass, which might translate to increased lipid content under certain conditions [10].

Besides that, salt stress significantly affects both the growth and nutrient composition of microalgae [11]. Salt stress affects the organic composition and growth of microalgae notably, leading to ionic imbalance, osmotic stress, and the creation of reactive oxygen species (ROS) that interfere with photosynthesis [11]. In response to salinity stress, microalgae have evolved distinct mechanisms, such as shifting from cell division to lipid storage [12]. Research on microalgae consistently highlights that optimizing one or two environmental conditions can greatly enhance their growth [13]. The objectives of this study were to investigate the impact of carbon source and salinity on the nutritional content and growth of *Chlorella* sp, and to characterize the biochemical profiles of microalgae as a source of biofertilizer.

2. Experiment

2.1 Pure microalgae strain and culture conditions

A wild-type strain of Chlorella sp., obtained from Prof. Dr. Lee Keat Teong at Universiti Sains Malaysia, was used in this study. The microalgae was cultivated and maintained in Bold's Basal Medium (BBM), prepared as follows: 10 mL/L of culture medium, which was made up of 25 g/L of $NaNO_3$, 2.5 g/L of $CaCl_2 \cdot 2H_2O$, 7.5 g/L of $MgSO_4 \cdot 7H_2O$, 7.5 g/L of K_2HPO_4, 17.5 g/L of KH_2PO_4, 2.5 g/L of NaCl; and 1 mL/L of culture medium which was consisted of 50 g/L of EDTA anhydrous, 31 g/L of KOH, 8.82 g/L of $FeSO_4 \cdot 7H_2O$, 1.44 g/L of $MnCl_2 \cdot 4H_2O$, 0.71 g/L of MoO_3, 1.57 g/L of $CuSO_4 \cdot 5H_2O$, and 0.49 g/L of $Co(NO_3)_2 \cdot 6H_2O$. The initial pH of the medium was adjusted to 3.0-3.5. Cultivation was performed in 100 mL conical flasks holding 50 mL of medium, containing 50 mL of medium, which were aerated with compressed air to sustain a CO_2 concentration of 10% v/v. The temperature was kept between 25–28°C, and the environment was continuously illuminated with cool-white fluorescent light (CWF, Philip TL-D 36 W/865) at the light intensity of 60–70 μmol $m^{-2}s^{-1}$. [14].

2.2 Cultivation of microalgae using chicken compost

Granular chicken manure fertilizer was acquired from a local market. A quantity of 10 g of the fertilizer was submerged in 600 mL of tap water and agitated using a magnetic stirrer for 24 hours. The composition of the chicken compost used in this study is published in the earlier publication [15]. The resultant fertilizer solution was then obtained by filtering the solid out of the solution using filter paper (Double Rings 101). Then, the fertilizer medium, 200 mL, was added to a photobioreactor containing 5 L of unsterilized tap water, and the pH of the of the final solution was tuned to a range of 3 to 3.5. Afterward, 500 mL of *Chlorella* sp., which was cultivated as seed culture, was added to the photobioreactor. The experiment was conducted with continuous aeration using compressed air and consistent illumination from cool-white fluorescent lights (CWF, Philip TL-D36W/865) at the light intensity of 60–70 $\mu mol\ m^{-2}s^{-1}$ [16].

2.3 Microalgae cultivation parameter studies- carbon sources

Microalgae cultivation followed the procedure outlined in Section 2.2, with the modification of substituting bubbling CO_2-compressed air with $NaHCO_3$ as the carbon source. A measured amount of $NaHCO_3$ was added to the cultivation medium to reach the concentrations of 0.1 g/L, 0.2 g/L, 0.3 g/L, and 0.4 g/L. The mixture was then stirred with a magnetic stirrer at 400 rpm, and the pH was adjusted to a range of 3.0 to 3.5 [17].

2.4 Microalgae cultivation parameter studies- salinity

Microalgae cultivation followed the protocol outlined in Section 2.2, with the exception of introducing varying concentrations of sodium chloride (NaCl) into the microalgae medium. The NaCl concentrations were adjusted from 0 M to 0.5 M in 0.1 M increments.

2.5 Microalgae growth assessment

A correlation was established between *Chlorella* sp. optical density and microalgal biomass. A Shimadzu UV mini-1240 spectrophotometer was used to measure the optical density of the culture at 688 nm every day. Subsequently, 10 mL of each sample underwent centrifugation for 5 minutes at 10000 g. The supernatant was removed, while the microalgal biomass was placed in an oven and heated at a temperature of 100°C for 24 hours. The analysis of samples was triplicated to secure data accuracy. [17]. The correlation is expressed in Equation (1):

$$\text{Dry weight (g/L)} = (0.532 \times OD688) + 0.0333,\ R^2 = 0.9720 \qquad (1)$$

2.6 Lipid extraction and measurement of lipid yield

30 mL vials were labelled and initially weighed. Then, 0.1 g of dried *Chlorella* sp. biomass was added to each vial containing 30 mL of Bligh and Dye solvent (methanol and chloroform in a 2:1 ratio). The mixture was agitated in an incubator shaker for 24 hours at room temperature. After filtration, the filtrate was left to air-dry in a fume hood for 24 hours to aid solvent evaporation. Subsequently, samples were dried in an oven at overnight at 105°C to ensure complete solvent removal. Finally, vials were weighed to determine the weight of extracted lipids [17].

https://doi.org/10.1051/e3sconf/202560301030

3. Results and discussion

3.1 Effect of different carbon sources on microalgae growth

Based on the findings depicted in Figure 1, CO_2 bubbling emerged as the superior carbon source, fostering a significant 5.60 % increase in microalgae growth compared to cultivation with sodium bicarbonate, with growth peaking at 1.32 g/L ± 1.2 %. When comparing various concentrations of $NaHCO_3$ in cultivation, 0.1 g/L of $NaHCO_3$ exhibited the highest microalgae growth at 1.25 g/L ± 0.7 %. Additionally, the growth of *Chlorella* sp. declined as the amount of $NaHCO_3$ added to the cultivation increased. This underscores that low concentrations of $NaHCO_3$ enhance microalgae growth, while high concentrations inhibit microalgae cell growth by exceeding the optimum carbon concentration [18]. Furthermore, as illustrated in Figure 1, the growth of *Chlorella* sp. cultivated with $NaHCO_3$ was generally slower and resulted in a lower microalgae concentration compared to cultivation with CO_2 bubbling. This slower growth could be attributed to environmental stress factors such as pH changes and altered carbonate chemistry caused by $NaHCO_3$. The introduction of $NaHCO_3$ can lead to increased alkalinity and fluctuations in bicarbonate and carbonate ion concentrations, disrupting the cellular homeostasis and biochemical pathways of *Chlorella* sp., making this cultivation method less favorable [18]. Given these results, CO_2 bubbling was selected for the subsequent studies.

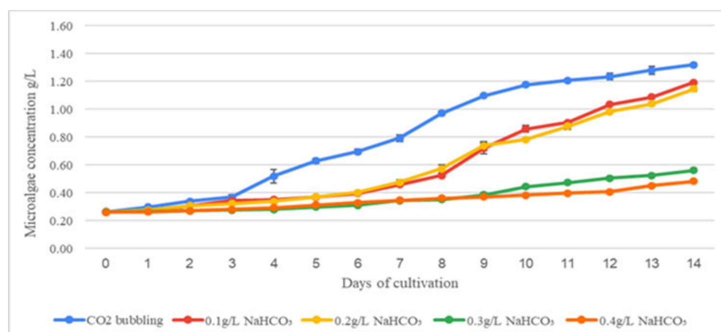

Fig. 1. The effect of different carbon sources on the growth of *Chlorella* sp.

3.2 Effect of salinity on microalgae growth

Salinity stress in cultivation medium can significantly impacts both the growth and organic composition of microalgae [10]. Analysis of the impact of NaCl concentrations variations on the growth of *Chlorella* sp. in Figure 2 revealed that 0.1 M exhibited the highest microalgae biomass production at 1.25 g/L ± 0.6%, closely resembling the one without NaCl (with the highest microalgae biomass recorded at 1.32 g/L ± 1.2%). However, as the NaCl concentration increased, the microalgae concentration of *Chlorella* sp. visibly declined. The lowest *Chlorella* sp. biomass production was observed at 0.5 M, with 0.63 g/L ± 3.5% as the highest peak. This decline can be attributed to elevated salinity levels, which prompt Na^+ to compete with Ca^{2+} for cell wall binding sites, reduce K^+ concentrations, disrupt cellular ion balance, and hinder protein synthesis and photosynthesis in microalgae cells [10]. Additionally, as shown in Figure 2, the growth of *Chlorella* sp. was directly proportional to the days of cultivation across all NaCl concentrations. There were no signs of inhibition or degradation, indicating that *Chlorella* sp. was tolerant to the highest 0.5 M NaCl concentration, although it exhibited the slowest growth compared to lower NaCl concentrations.

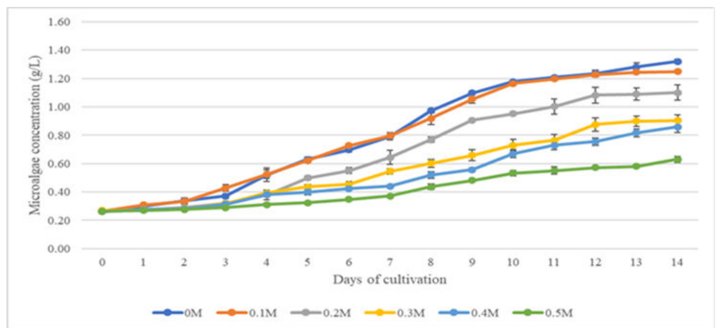

Fig. 2. The impact of NaCl concentration on the growth of *Chlorella* sp. under CO_2 bubbling cultivation.

3.3 Lipid content and lipid yield

The result of lipid accumulation in in *Chlorella* sp. under CO_2 bubbling cultivation is shown in Figure 3. *Chlorella* sp. subjected to 0.5 M NaCl exhibited the highest lipid content at 34% ± 1.8%, whereas the lowest content occurred at 0.1M, with 18% ± 2.8%. However, despite having the highest lipid content, *Chlorella* sp. in 0.5M salinity cultivation did not exhibit the highest lipid yield, with only 0.21 g/L ± 5.0 %, due to having the lowest microalgae biomass production; while the highest lipid yield is recorded at medium without NaCl, with 0.42 g/L ± 3.0 %, due to having the highest microalgae biomass production. These results aligned with the findings of Singh, et al., indicating that salt stress can significantly enhance lipid accumulation in microalgae; however, high salinity also inhibits microalgae growth, which ultimately leads to a decrease in overall lipid production. [19]. However, *Chlorella* sp. exhibited unusual behavior at a 0.3 M NaCl concentration, showing the lowest lipid yield at 0.18 g/L ± 2.9%. At this concentration, the osmotic stress was too severe for the cells to grow effectively, thus insufficient to trigger a strong lipid accumulation response. This resulted in reduced growth and lower overall lipid production [11]. In contrast, at elevated NaCl concentrations, the severe osmotic stress triggered mechanisms that led to lipid accumulation as a protective measure, resulting in higher lipid content despite reduced growth, which were shown in 0.4M and 0.5M [11]. This is due to the increased salinity caused *Chlorella* sp. to prioritize lipid accumulation over cell division to cope with the salt stress in the cultivation environment [12].

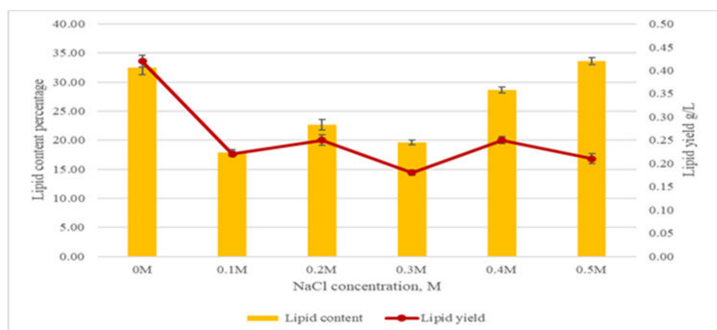

Fig. 3. The effect of NaCl concentration on the lipid content and lipid yield of *Chlorella sp.*

4. Conclusion

By refining the cultivation techniques for *Chlorella* sp., it is possible to achieve maximum growth and lipid production, addressing challenges in large-scale biofertilizer production. This study explored the impact of carbon sources and salinity on enhancing biomass production and lipid accumulation in *Chlorella* sp. The results showed that *Chlorella* sp. grows best in a medium with compressed CO_2 bubbling as the air supply, achieving the highest microalgae concentration of 1.32 g/L ± 1.2%. Regarding salinity under CO_2 bubbling, the study showed that *Chlorella* sp. reached optimal microalgae concentration and lipid yield in a medium without NaCl, with values of 1.32 g/L ± 1.2% and 0.42 g/L ± 3.0 %, respectively.

The authors would like to acknowledge the funding from Joint Research Project between Curtin-UTP-Nottingham-UMT (cost centre 015MD0-187), Ministry of Higher Education (MOHE) Malaysia through HICoE Grant (cost centre 015MA0-052) to CBBR and Yayasan Universiti Teknologi PETRONAS (cost centre 015LC0-410).

References

1. S. P. Tan, L. C. Ng, N. Lyndon, Z. Aman, P. Kannan, K. Hashim, A review on post-COVID-19 impacts and opportunities of agri-food supply chain in Malaysia. Peer J. **11**, e15228 (2023). https://doi.org/ 10.7717/peerj.15228

2. A. Barros-Rodríguez, P. Rangseekaew, K. Lasudee, W. A.-O. Pathom-Aree, and M. A.-O. Manzanera, Impacts of Agriculture on the Environment and Soil Microbial Biodiversity. Plants. **10**, 2325 (2021). https://doi.org/10.3390/plants10112325

3. S. S. Ali, M. Kornaros, A. Manni, R. Al-Tohamy, A. E.-R. R. El-Shanshoury, I. M. Matter, Chapter 28 - Advances in microorganisms-based biofertilizers: Major mechanisms and applications. Biofertilizer. **1**, 371-385 (2021). https://doi.org/10.1016/j.biortech.2021.124870

4. N. Fazelian and M. Yousefzadi, Chapter 8 - Nano-biofertilizers for enhanced nutrient use efficiency, in Nano-enabled Agrochemicals in Agriculture. AP. 145-158 (2022).

5. S. Nosheen, I. Ajmal, and Y. Song, Microbes as Biofertilizers, a Potential Approach for Sustainable Crop Production. Sustainability. **13**, 1868 (2021). https://doi.org/10.3390/su13041868

6. N. Rasdi, A. Ramlee, E. Wahid, and M. Jusoh, Microalgae and the factors involved in successful propagation for mass production. J. Environ. Sustain. **16**, 21-42 (2021).

7. E. Daneshvar, Y. Sik Ok, S. Tavakoli, B. Sarkar, S. M. Shaheen, H. Hong, Insights into upstream processing of microalgae: A review. Bioresour. Technol. **329**, 124870 (2021). https://doi.org/10.1016/j.biortech.2021.124870.

8. X. Ma, Y. Mi, C. Zhao, and Q. Wei, A comprehensive review on carbon source effect of microalgae lipid accumulation for biofuel production. Sci. Total Environ. **1**, 804 (2022). https://doi.org/10.1016/j.scitotenv.2021.151387

9. S. Fal, A. Aasfar, R. Rabie, A. Smouni, and H. E. L. Arroussi, Salt induced oxidative stress alters physiological, biochemical and metabolomic responses of green microalga Chlamydomonas reinhardtii. Heliyon. **8**, e08811(2022).

10. N. Haris, H. Manan, M. Jusoh, H. Khatoon, T. Katayama, and N. A. Kasan, Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species. Aquacult. Rep. **22**, 100925 (2022). https://doi.org/10.1016/j.aqrep.2021.100925
11. P. Shetty, M. M. Gitau, and G. A.-O. Maróti, Salinity Stress Responses and Adaptation Mechanisms in Eukaryotic Green Microalgae. Cells. **8**, 1657 (2019).
12. M. E. H. Osman, A. M. Abo-Shady, S. F. Gheda, S. M. Desoki, and M. E. Elshobary, Unlocking the potential of microalgae cultivated on wastewater combined with salinity stress to improve biodiesel production. Environ Sci Pollut Res. **30**, 114610–114624 (2023). https://doi.org/10.1007/s11356-023-30370-6
13. A. Josephine, T. S. Kumar, B. Surendran, S. Rajakumar, R. Kirubagaran, and G. Dharani, Evaluating the effect of various environmental factors on the growth of the marine microalgae, Chlorella vulgaris. Front. mar. sci. **9**, 954622 (2022). https://doi.org/10.3389/fmars.2022.954622
14. K. Kumaran, M. Lam, X. Tan, Y. Uemura, J.-W. Lim, C. G. Khoo, Cultivation of Chlorella vulgaris Using Plant-based and Animal Waste-based Compost: A Comparison Study. Procedia Eng. **148**, 679-686 (2016). https://doi.org/10.1016/j.proeng.2016.06.551
15. U. Suparmaniam, M. K. Lam, J. W. Lim, H. Rawindran, Y. C. Ho, I. S. Tan, Enhancing high-density microalgae cultivation via exogenous supplementation of biostimulant derived from onion peel waste for sustainable biodiesel production. J. Environ. Manag. **359**, 120988 (2024). https://doi.org/10.1016/j.jenvman.2024.120988
16. U. Suparmaniam, M. K. Lam, Y. Uemura, S. H. Shuit, J. W. Lim, P. L. Show, Flocculation of Chlorella vulgaris by shell waste-derived bioflocculants for biodiesel production: Process optimization, characterization and kinetic studies. Sci. Total Environ. **702**, 134995 (2020). https://doi.org/10.1016/j.scitotenv.2019.134995
17. M. K. Lam and K. T. Lee, Effect of carbon source towards the growth of Chlorella vulgaris for CO2 bio-mitigation and biodiesel production. Int. J. Greenhouse Gas Control. **14**, 169-176 (2013). https://doi.org/10.1016/j.ijggc.2013.01.016
18. J. Li, C. Li, C. Q. Lan, and D. Liao, Effects of sodium bicarbonate on cell growth, lipid accumulation, and morphology of Chlorella vulgaris. MCF. **17**, 111 (2018).
19. R. A.-O. Singh, P. Yadav, A. A.-O. Kumar, A. Hashem, G. A.-O. X. Avila-Quezada, E. A.-O. Abd Allah, Salinity-Induced Physiochemical Alterations to Enhance Lipid Content in Oleaginous Microalgae Scenedesmus sp. BHU1 via Two-Stage Cultivation for Biodiesel Feedstock. Microorganisms. **11**, 2064 (2023). https://doi.org/10.3390/microorganisms11082064